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FUNCTIONALITY OF GAS PRECIPITATED CASEIN AND THE IMPLICATION FOR THE CREATION OF SURIMI-LIKE SEAFOOD ANALOGS

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INTRODUCTION

Milk Proteins are highly desirable as ingredients in foods. In addition to their availability and high nutritive value, their utility is enhanced by their bland, almost transparent, flavor. Casein, the major protein component of cow's milk, and its caseinate derivatives, have physicochemical, functional and nutritive properties which make them useful worldwide (Southward, 1985). High concentrations of caseins have exceptional water binding capacity, fat emulsification properties, whipping ability, and are viscous and soluble in neutral or alkaline conditions (Jonas et al., 1976) .

Conventional precipitation of casein from skim milk is achieved through the addition of rennet or by acidification to the isoelectric point (pH = 4.6). Production of rennet casein (pH = 6.6) incorporates an enzyme shearing of the protein molecules. Acid casein involves the addition of a mineral acid, sulfuric or hydrochloric, or by the inoculation with a bacteria that produces lactic acid from the lactose.

Other methods of precipitating proteins include salting out using a salt such a calcium chloride, the addition of a weak polar solvent such as ethanol and the addition of metal ions (e.g., calcium). The acid precipitation of casein by dissolution of CO_2 in milk was demonstrated by Jordan et al. 1987. Casein precipitation takes place when carbon dioxide at pressures greater than 100 psi is dissolved in milk (Jordan et al. 1987). Casein obtained in this manner has chemical properties between those of acid casein produced by mineral acid or the action of lactic acid producing bacteria and rennet casein. These properties are directly related to the precipitation pressure and temperature. The whey product is a sweet whey with a pH of 6.3 as opposed to acid whey with a pH of 4.6. When carbon dioxide is dissolved in milk, carbonic acid is formed by the reaction between carbon dioxide and the aqueous portion of the milk. The reversible reactions for the dissolution of carbon dioxide in water are:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+$$

ppm Ca^{2+} . The analysis of the milk was within standard deviations reported for milk (Jenness, 1988 and McBean and Speckman,1988).

The casein was precipitated at 5520 kPa and 38°C for five minutes in a carbon dioxide batch pressure chamber apparatus. Three batches per day for three days were prepared and processed for a total of nine casein samples. Batches were selected for inclusion in the study only when recovery was sufficient for complete analysis and the product met microbiological standards for grade "A" milk standards and dry milk standards. The casein from each batch selected was used wet for the proximate analysis and solubility studies; the remainder of each batch (55 to 90 g) was suspended in about 400 ml of reagent grade water using a Polytron homogenizer and freeze dried. The freeze dried caseins were used for the foaming and emulsion studies.

Acid casein was also prepared from raw skim milk by isoelectric precipitation at pH 4.6 at 30°C with 1 M hydrochloric acid. The curd was separated by filtering through four layers of cheese cloth and washed once with 4X the volume of the milk. The curd was resuspended in a volume of reagent grade water equal to the original volume of the milk and the pH was adjusted to 7.0 with 1 M Sodium Hydroxide. The solution was filtered through Whatman #541 paper and the entire sequence repeated. The casein solution was then precipitated again at pH 4.6 with 1 M Hydrochloric acid, separated, washed once, resuspended in water and freeze–dried. Sodium caseinate was prepared in a similar manner except that the 3X washed casein was dissolved at pH 7.0 prior to freeze–drying. The commercial caseins evaluated were Alacid™ , an edible lactic casein, Alanate™ 310, a calcium caseinate, and Alanate™ 130, a granular sodium caseinate. The proximate analyses of these products are shown in table 1.

Proximate Analysis

Moisture (solids), ash (AOAC, 1984) and calcium were determined on the wet CO_2-casein and milk samples.

Bacterial Analysis

Bacteriological analysis for total plate count and coliforms were carried out on the milk and each of the wet casein samples as described by Houghtby et al., 1992. Analysis for yeast and molds was adapted from the method of Houghtby et al. (1992) for pour plates by substituting potato dextrose agar which is advantageous for the growth of yeast and mold for total plate count agar.

Solubility

Solubility as a function of pH in both water and .1 M sodium chloride was measured by corrected absorbance at 280 nm (Strange et al., 1994) with the following adaptations. The pH of the CO_2- precipitated casein dissolved in either water or .1 M sodium chloride was expected to be lower than commonly found for sodium caseinates. Samples of known weight of the wet casein (approximately .8 g in 100 g of water or .1 M sodium chloride) were titrated with both 1 M sodium hydroxide to raise the pH to 7.0 and .1 M hydrochloric

$$EAI = \{(2.303 \times 2 \times A_{500} \times \text{dilution factor})/(c \times [1 - \text{oil volume}] \times 10{,}000)\}$$

where A_{500} = absorbance at 500 nm, and c = grams of protein per milliliter of aqueous solution before emulsion. Emulsion stability was the time, in minutes, for the turbidity of the emulsion to decrease 50%. Each emulsion was made in duplicate and repeated three times. It was assumed for calculation purposes that all of the casein was soluble at pH 7.

Statistical Analysis

ANOVA and other statistical tests such as student–t test were carried out as described on Steel and Torrie, 1980.

RESULTS AND DISCUSSION

A 2^4 factorial experimental design was used to screen for the effects of temperature, pressure quantity of milk and residence time on casein yield and curd appearance. The levels used for the study are shown in table 2. The results of this study are shown in figure 3 as a probability plot which was used because the factorial experiment was unreplicated. The probability plot accounts for real and meaningful, high order interactions. As can be seen the relationship is linear and deviations from linearity indicate parameters that are not easily explained by chance occurrence. The main effects of milk quantity, ant the interactions of pressure X temperature, pressure X quantity, temperature X quantity and the three order interaction of pressure X quantity X temperature all impacted on casein yield. The effects of residence time, within the range studied, were not significant. The screening study also showed that the curd appearance was influenced by temperature. At 38°C and at either pressure, the curd was moist and cottage cheese-like. The curtd was watery only when precipitation was less than 90% complete. Samples precipitated at temperatures between 32-49 °C with yields greater than 90% had discernable particles whereas at higher temperatures the curd was dry and had a rubbery feel with no evidence of individual particles. The dry and rubbery appearance is evidenced by an analysis of solids content shown in table 3 where moisture loss at all pressures is apparent as temperature increases.

Figure 4 shows the effect of milk quantity on normalized casein yield. The yield increased to 100% at temperatures greater than 38°C and a pressure of 4140 kPa as the milk sample size was increased from 200 to 750 grams and then decreased sharply with further increases in sample size. Figure 5 presents normalized yield as a function of precipitation pressure and temperature. Increasing pressure results in an increase in yield.

Based on these studies the following conditions were used for the functionality study: (5520 kPa; 38°C and five minute residence time using a 750 gram sample).

The reactor was cleaned and sanitized after each run and all samples were analyzed at various stages of the handling process to ensure compliance with dry milk standards.

in the fines. At pH below the isoelectric point, the protein redissolved until it was completely soluble at a pH of 3.1 or lower (figure 7).

The foaming ability and stability of the CO_2-caseins are listed in table 5 as well as those of the commercially and laboratory prepared caseins. The % overrun found for the CO_2-caseins was not significantly different ($P > .05$) than that found for the laboratory prepared acid casein. The % overruns for the laboratory prepared sodium caseinate and the commercial caseins were significantly higher than those of the CO_2-casein, but the foam stability of the CO_2-casein was significantly better. This increased foam stability is probably related to the high foam density which encourages increased drainage stability. The % overrun increased with increased whipping time for all caseins; these increases have been attributed to the resistance of casein to denaturation and aggregation.

Emulsification activity index (EAI) of the CO_2-caseins was significantly higher than that of a commercial lactic acid casein but significantly lower than that of a commercial sodium caseinate. These differences are probably due to differences in solubility even though the pH values of these solutions were all adjusted to 7 since it has been reported that undissolved protein contributes little to emulsification.

Emulsion stabilities (ES) of the CO_2-caseins was greater than any of the commercial caseins. These differences occurred despite adjustment of the pH to 7 in all cases. Examination of the data in table 6 shows two groupings in the ES data. The differences observed may be due to the heat treatment received by the commercial samples which were spry dried rather than freeze dried.

CONCLUSIONS

The CO_2 precipitation process produced caseins which were consistent. The were partially insoluble at pH 7; this insolubility affected the functional properties when compared with either acid casein or sodium caseinate. The functional behavior of the CO_2–caseins suggests that the calcium is complexed in a different manner than that found in commercial calcium caseinate. CO_2-caseins produced the most stable foams and emulsions, although they did not have the greatest whippability or the highest emulsion activity index.

The physical properties of the CO_2-caseins can be altered by changes in the pressure and temperature of the reaction. Additional work is planned to explore the effect these changes have on the functional properties.

Table 1. Proximate Analysis of Commercial Caseins.

Casein		% Protein	% Moisture	% Ash	% Fat	% Lactose	pH(5 % Sol)
Alacid™		87.3	9.6	1.8	1.3	.1	4.6
Alanate™	310	83.5	10.1	4.3	1.1	.1	6.7
Alanate™	130	90.5	4.2	4.1	1.1	.1	7.3

Table 2. Factorial Experimental Design

Pressure (kPa)	2760	5520
Temperature (°C)	38	60
Residence Time (min)	5	10
Milk Quantity (g)	500	750

Table 5. % Overrun for caseins after 5, 10, and 15 minutes whipping and time in minutes for 50% collapse of the foam.

Whip Time (min)	CO_2-CN	Acid-CN	Na-CN	Alacid™ 710 Lactic-CN	Alanate™ 130 Na-CN	Alanate ™ 310 Ca-CN
5	270	278	487**	545**	634**	773**
10	341	345	557**	581**	686**	835**
15	397	511**	645**	586**	727**	868**
Time (min) for 50% foam collapse						
	28.6	20.0*	1.1**	17.9**	10.2**	15.8**

* $P < .05$ that % overrun of CO_2-casein and standard casein or collapse time of CO_2-casein and standard casein are the same (t-test)

** $P < .01$ that % overrun of CO_2-casein and standard casein or collapse time of CO_2-casein and standard casein are the same (t-test)

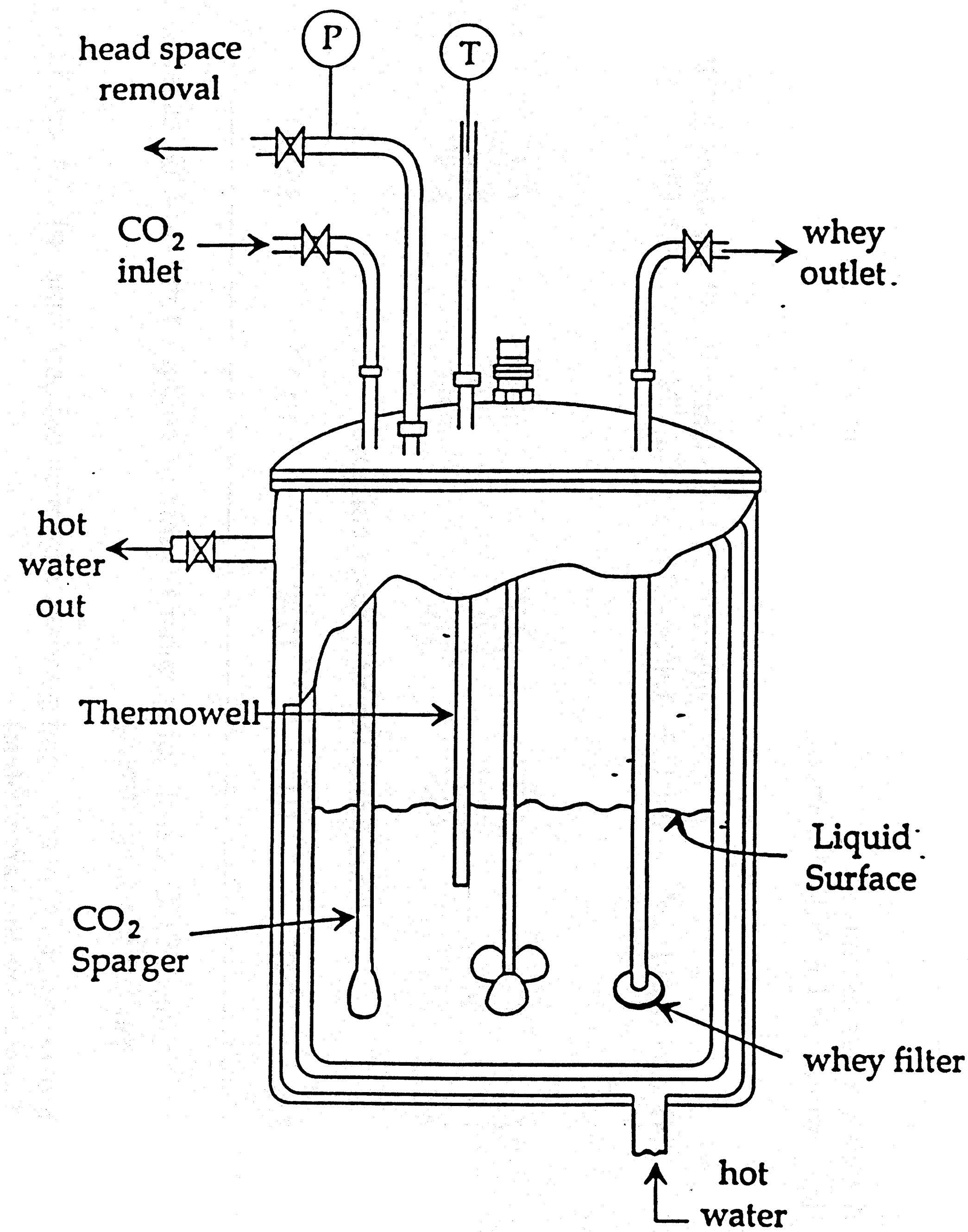

FIGURE 1. BATCH REACTOR

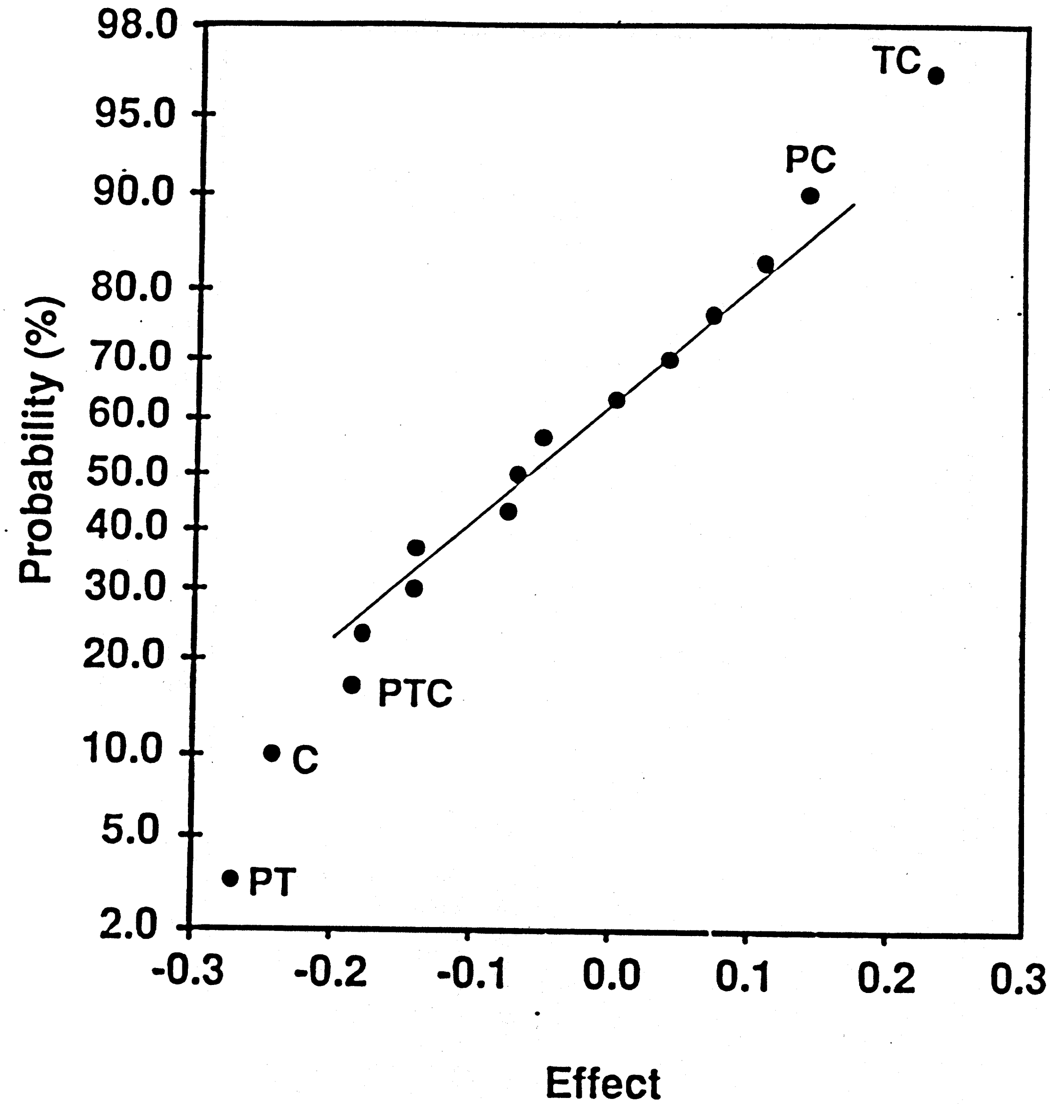

FIGURE 3. PROBABILITY PLOT OF DESIGN PARAMETERS (MAIN EFFECTS AND INTERACTIONS)
(P – PRESSURE; T – TEMPERATURE; R – RESIDENCE TIME; C – MILK QUANTITY)

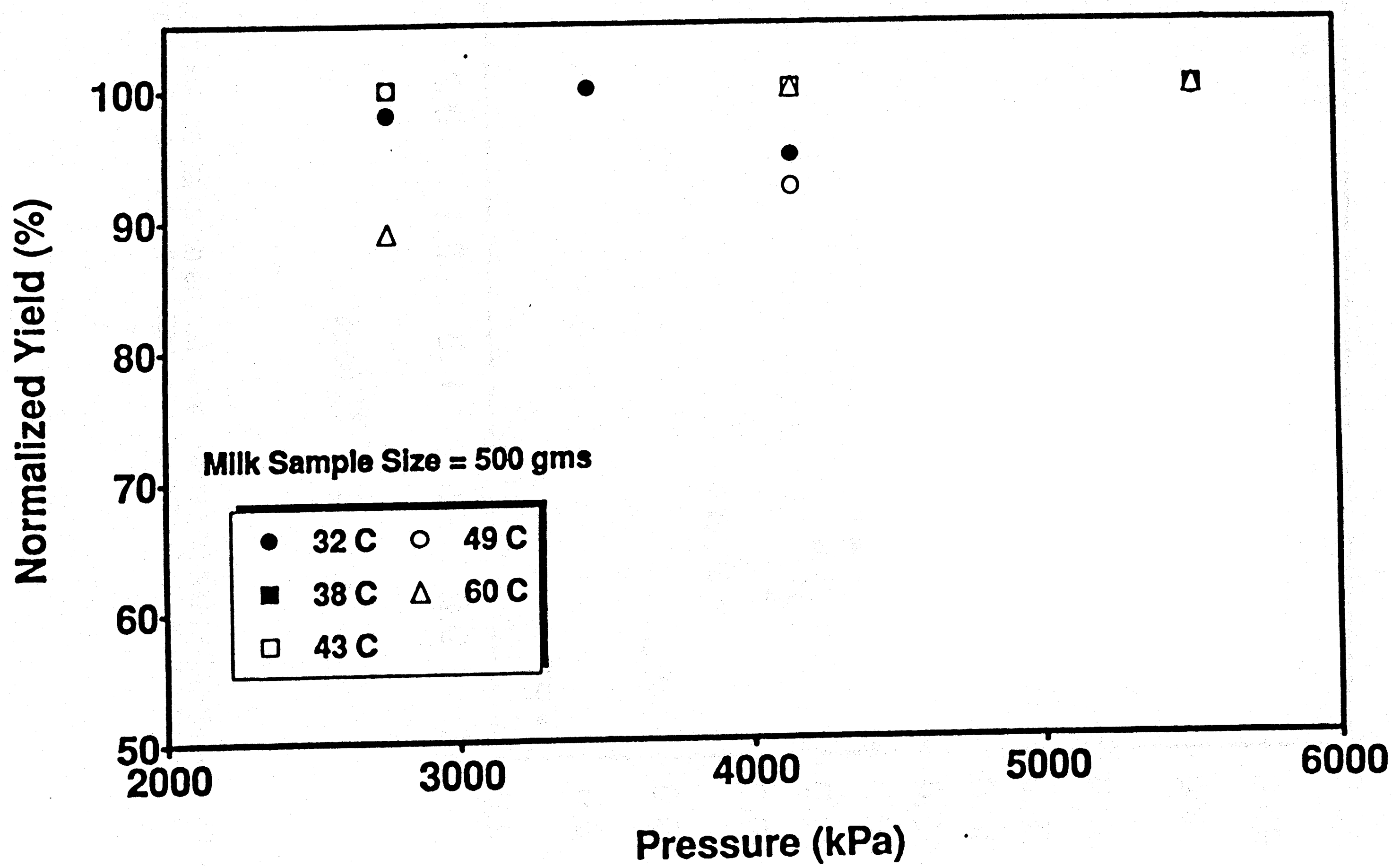

FIGURE 5. Casein Yield as a Function of Temperature and Pressure.

FIGURE 7. SOLUBILITY OF GAS–PRECIPITATED CASEIN FINES IN WATER AS A FUNCTION OF pH